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## INHIBITION OF DIVISION AND DEVELOPMENT OF SEA-URCHIN EGGS BY ANTISERA AGAINST FERTILIZIN\*

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In the previous paper<sup>1</sup> it was reported that rabbit antisera prepared against fertilizin of sea urchins were effective in blocking cell division and development of the fertilized eggs. The results of some further investigations of this action are presented here. The materials and testing methods were the same as previously described, except where otherwise noted.

### EXPERIMENTS

*Development in Immune Sera.*—As noted in the previous paper, rabbit antisera prepared against the fertilizins of the sea urchins *Strongylocentrotus purpuratus* and *Lytechinus pictus* can block division before the first cleavage when added to the demembranated eggs a short time after fertilization. The amount of developmental progress attained is a function of the strength of the antiserum. An antiserum that blocks division before the first cleavage when used full strength will, upon dilution, permit development to proceed to progressively later stages of cleavage and embryonic development.

Cytological studies have been made of eggs that have been treated with strong antiserum at various times in the division cycle. These have shown that nuclear as well as cytoplasmic division is arrested and that mitosis may be stopped at practically any stage of the cycle, there being no evidence of a specially sensitive phase. The strongest antisera so far available, permit, when used full strength, an amount of development equivalent to approximately one-fifth to one-fourth of a division cycle. Thus, for example, eggs of *Lytechinus* that take 95 minutes for the first division at 17° C. will make about 20 minutes' developmental progress in an undiluted

strong fertilizin-antiserum regardless of what time after fertilization they are introduced. If placed in the antiserum less than 20 minutes before the first cleavage, they can complete this division. Antisera of this strength can be diluted 16- to 32-fold and still block the first division if the eggs are introduced less than 10 minutes after fertilization. No marked changes are observed initially in eggs whose division has been blocked by antiserum. After a time, however, cytolytic effects become evident, as shown in Figure 1. The time at which cytolytic changes are observed is also a function of the strength of the antiserum. In the strongest sera this can occur within  $2\frac{1}{2}$  hours in the two species investigated.

A relatively short exposure to strong antiserum suffices to block division irreversibly. In a series of six experiments with eggs of *S. purpuratus*, placed in undiluted antiserum (capable of blocking first cleavage when diluted 16-fold) 10–15 minutes after fertilization, exposures of 30 minutes completely inhibited division. With exposures shorter than 15 minutes, normal development ensued, and between 15 and 30 minutes there was partial inhibition.

As noted in the previous paper, antisera that block division in early cleavage stages also inhibit the development of embryos introduced at later stages. No detailed investigation has, as yet, been made of the effect on cell division in embryos in blastula and later stages, but a number of the cleavage-blocking antisera have been titrated with respect to their cytolytic and lethal action on the embryos in blastula, gastrula, prism, and pluteus stages. Comparison of the results of the tests on the embryos with those on the uncleaved eggs showed no significant difference, with allowance for about twofold range of variation, in the serum dilutions and time in which the lethal effects were obtained.

**Absorption Experiments.**—Most of the fertilizin-antisera were found to possess agglutinins for the homologous sperm. Thus, of 23 cleavage-blocking antisera against fertilizin of *S. purpuratus*, 17 agglutinated sperm with titers (on 1 per cent suspension) ranging from 2 to 128. There was no evident correlation between sperm-agglutinating action and cleavage-blocking effect, especially as the latter effect was exhibited also by sera possessing no demonstrable sperm agglutinins. However, in view of the possibility that nonagglutinating (“univalent”)<sup>2</sup> antibodies might be involved, cleavage-blocking tests were made with sperm-absorbed antisera. In ten tests, cleavage-blocking activity remained after removal of detectable sperm agglutinin. In three of these, a reduction of blocking activity was noted. The maximum reduction obtained may be illustrated by the results of an experiment with an *S. purpuratus* fertilizin-antiserum that had an agglutination titer of 128 on sperm. Absorption with homologous sperm removed detectable sperm agglutinin. When the antiserum was titrated on fertilized uncleaved eggs, the following results were obtained for the maximum development obtained in 3 days:

	DILUTION					
	2× to 16×	32×	64×	128×	256×	512×
Unabsorbed	1-cell†	1-, 2-†	4-†	16-†	32-, 64-†	Mostly bottom-swim. blast.†
Absorbed	1-cell†	2-, 4-†	4-, 8-†	16-, 32-†	64-, blastula	Top and bottom-swim. blast. and gast.

The dagger (†) indicates that cytolysis has taken place. In this experiment the reduction in cleavage-blocking action is approximately the equivalent of that which

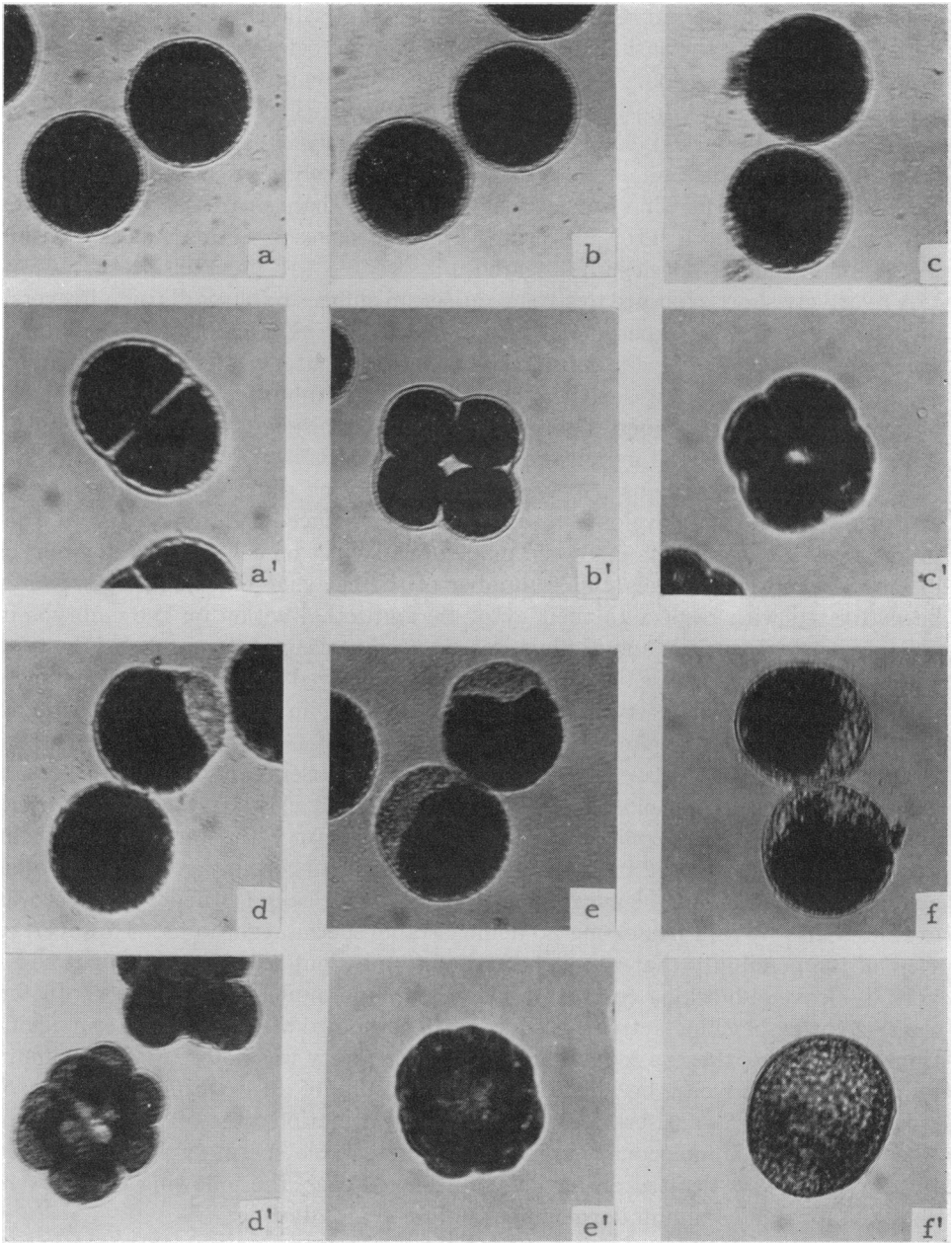


FIG. 1.—Inhibition of division of fertilized, demembrated eggs of the sea urchin *Strongylocentrotus purpuratus* by treatment with a rabbit antiserum against homologous fertilizin. *a-f*: antiserum-treated eggs; *a'f'*: controls at the same time of development. Eggs placed in antiserum at 15 minutes after fertilization and photographs taken at 2(*a, a'*), 3(*b, b'*), 4(*c, c'*), 5(*d, d'*), 6(*e, e'*), and 26(*f, f'*) hours (at 17° C.) after fertilization. Magnification,  $\times 175$ .

would be obtained by dilution of the antiserum to about 75 per cent. This would correspond to the removal of about 25 per cent of the blocking antibodies. The results of two other experiments showed somewhat less effect, and the remaining seven showed no significant reduction.

As previously noted,<sup>1</sup> antisera against sperm, or against antifertilizin, have no cleavage-blocking action. These antisera agglutinate sperm with titers in many cases greater than 1,000. Evidently the antibodies against sperm do not have cleavage-blocking action. One may, then, interpret the reduction of cleavage-blocking action, in certain fertilizin-antisera after absorption with sperm, to mean that some of the antibody molecules in these sera are capable of reaction with both sperm and eggs.

The cleavage-blocking action of the fertilizin-antisera can be completely removed by absorption with unfertilized eggs and by fertilized, demembranated eggs in early cleavage stages. Antisera that have a titer of 16-32, with respect to blocking of the first cleavage, are rendered noninhibiting when absorbed for about 1 hour with an equal volume of a 50 per cent suspension of the eggs.

Antisera have been absorbed also with living embryos. In tests on uncleaved eggs, a considerable reduction of cleavage-blocking action was found. Thus, in one detailed experiment with an antiserum against fertilizin of *S. purpuratus*, three successive 1-hour absorptions with a total of 0.9 volume of packed gastrulae were required to remove the immobilizing and cytolytic action of the antisera on these embryos. In tests on the uncleaved eggs, the following results were obtained for developmental progress in two days:

	DILUTION							
	4×	8×	16×	32×	64×	128×	256×	512×
Unabs.	1-cell†	1-cell†	1-, 2-†	4-, 8-†	Ca. 32-†	Blas- tula†	Early Gas- trula	Gastrula
Absorbed	Ca. 32- cell†	Early blas- tula†	Blas- tula†	Early gas- trula	Gastrula	Gastrula	Gastrula	Gastrula

The absorption with gastrulae evidently removed a large amount, but not all, of the cleavage-blocking antibodies. It can be estimated, in this experiment, that about 10 per cent of the blocking activity remains after the absorption.

*Precipitation Tests.*—In the cleavage-blocking antisera the fertilized eggs exhibit, after a period of time, a dark irregular outline on the surface of the ectoplasmic layer, and later a distinct membrane (precipitation membrane) appears. The reaction of the antisera with material of the ectoplasmic layer is further shown with solutions of the latter. Such solutions, prepared by extracting suspensions of fertilized, demembranated eggs with Ca-free artificial sea water, give distinct precipitates when mixed with cleavage-blocking antisera. No precipitate was obtained with normal serum or antiserum that had been absorbed with fertilized eggs so as to remove cleavage-blocking activity.

*Effect on Tension at the Surface.*—When eggs are centrifuged at fairly high speeds, they become elongated and constrict into two fragments across the axis of stratification. Determination of the forces required for such separation has been used<sup>3</sup> as a method for estimating tension at the surface. Tests with unfertilized, jellyless eggs

in antiserum showed an increase of approximately 40 per cent, over eggs in normal serum, in the force necessary to separate the egg into light and heavy halves.

*Oxygen Consumption of Antiserum-treated Eggs.*—Measurements of the rate of uptake of oxygen showed a marked temporary increase when cleavage-blocking antiserum was added to suspensions of unfertilized eggs. In experiments with eggs of *S. purpuratus*, antiserum or control serum was added to the eggs from the side arm of the Warburg flasks at 60 minutes after fertilization (18° C.). The rate in antiserum increased to a maximum at 20–40 minutes that was about 4.5 times the rate of the controls and then returned to the control value during the next 40 minutes. The total O<sub>2</sub> consumption by the antiserum-treated eggs during the 80-minute period was 2.5 times that of the eggs in control serum.

*Sodium Content of Antiserum-treated Eggs.*—It is known<sup>4</sup> that sea-urchin eggs have the same sort of unequal distribution of certain ions with respect to the medium as do many other types of cells, Na<sup>+</sup> and Cl<sup>−</sup> being very low inside the egg and K<sup>+</sup> very high. Tests were therefore made to determine whether or not this distribution was upset by treatment with antiserum. The determinations have, so far, been made on Na<sup>+</sup>, by a colorimetric method<sup>6</sup> with fertilized eggs of *S. purpuratus* treated with blocking antiserum and control serum. The eggs were packed by centrifugation after 2 hours in the sera, cytolysed in distilled water, and aliquots analyzed after deproteinization. While precautions were taken to insure that experimental and control tubes of packed eggs contained close to the same amount of supernatant and interstitial fluid, the relatively high Na<sup>+</sup> content of the latter served to make this the largest source of error. The results of a triplicate set of experiments showed that within a margin of error of some 20 per cent there was no increase in the Na<sup>+</sup> content of the antiserum-treated eggs.

#### SUMMARY

Rabbit antisera against fertilizin of sea urchins can block cell division in the early cleavage of the egg and can also inhibit the development of embryos introduced at later stages. The blocked eggs and the embryos later undergo cytolysis in the immune sera, the dilutions and times at which this effect is obtained being similar for all stages investigated. Nuclear as well as cytoplasmic division is inhibited by the antiserum. Mitotic progress in strong antiserum is less than one-fourth of a division cycle, with no indication of a selective action on a particular mitotic phase. Swimming embryos in blastula to pluteus stages are almost immediately immobilized by strong antiserum. Absorption with sperm generally removes no detectable amount of cleavage-blocking antibodies but in some cases may reduce the titer by 25 per cent. Absorption with gastrulae can remove about 90 per cent of the cleavage-blocking antibodies. Precipitin tests show a reaction of the blocking antisera with extracts of the ectoplasmic layer of the fertilized eggs.

Treatment with antiserum increases the tension at the surface of the egg. The rate of oxygen uptake is temporarily increased by treatment of the fertilized eggs with blocking antiserum. Determination of the sodium content of the eggs shows no significant increase upon treatment with antiserum.

The cell division-blocking action of the fertilizin-antisera may be attributed to an initial reaction with surface constituents of the demembranated fertilized eggs that

bear antigenic structures in common with fertilizin and that persist, for the most part, in the later embryos.

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